IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Serial No. 10/677,733	Customer No.: 23379
Applicant: Gardner et al.	Confirmation No. 4887
Filed: Oct 01, 2003	Group Art Unit: 1656
Docket No. UTSD:1510	Examiner: Nashed, Nashaat T.

Title: NMR Detection of Foreign PAS Domain Ligands

DECLARATION UNDER 37CFR1.132

I, Professor Kevin H. Gardner, declare and state as follows:

1. I am an Associate Professor of Biochemistry and Pharmacology at the University of Texas Southwestern Medical Center in Dallas, where I also serve as the Chairman of the Molecular Biophysics Graduate Program. The Board of Regents of the University of Texas System is the assignee of this patent application. I have authored numerous scientific papers in the field of NMR analyses of protein structure, function and regulation. I am a coinventor on this patent application, and have read and considered the Final Action dated Dec 11, 2007, and Declaration of Professor Stephen Sprang dated Nov 19, 2007.

2. As someone skilled in the general area of biophysics, with over twenty years of expertise, predominantly in the area of NMR spectroscopy, I concur with the statements and opinions of Professor Sprang as provided in his Declaration dated Nov 19, 2007.

3. Furthermore, based on my expertise and carefully consideration, it is my conclusion that the commentaries about protein analysis put forth in the Final Action are not reflective of ordinary skill and understanding in the art. Its conclusions are unwarranted and erroneous, and its analysis contains multiple and fundamental overstatements and technical inaccuracies.

4. As detailed below, the Action fundamentally relies on two suppositions, both of which are technically incorrect: (a) that it is obvious to screen PAS domains because they all contain ligand binding sites; and (b) that solution NMR is an obvious choice of screening method to identify protein-binding ligands for any target.

5. "The ordinary skill in the art would have known of the presence of the ligand-binding cavity because the protein has an activity in solution." Action, p. 3.

This statement is both misleading and inaccurate. Indeed, most known PAS domains are not

naturally regulated by small molecule ligands or cofactors, and thus their activities in solution do not involve the binding of any such compounds. Examples include HERG (e.g. Cabral 1998), ARNT (e.g. Reisz-Proszasz et al., Mol Cell Biol 14(1994): 6075), and many others. The art teaches that these domains function as constitutive protein/protein interaction domains in their current settings, independent of natural small molecule regulation. Our innovation has been to establish a general way to test if these proteins might still be able to bind foreign core ligands despite their native disposition.

6. "[Whitty et al., 1997] takes the view that developing modulator protein activity involving protein/protein interaction is difficult, but not impossible. … [and Tiliey et al. 1997 provide] expectation of success to one of ordinary skill in the art that modulators of protein/protein interaction are possible to identify." Action, p. 4.

The accepted view in the field is that developing protein/protein inhibitors is difficult, but not always impossible, as shown by Tiliey 1997. But we are targeting internal binding sites that will have allosteric control of protein binding/activity, not the commonly-used route of directly binding the external protein-binding surface. Core targeting raises several additional challenges which made it a non-obvious route for an ordinarily skilled worker in the field:

- without an a priori formed cavity there would be an overwhelming expectation that our targeted core ligand binding sites would not even exist;
- it would be uncertain and unpredictable whether ligand binding to interior sites would be able to provoke an allosteric change that affects protein function, as opposed to the more straightforward options provided for exterior sites (e.g. direct occlusion of an external site is guaranteed to block function).
- compounds that target internal sites will likely have slow rates of binding ("on rates"), with correspondingly lower affinities than compounds which target the exterior surfaces.

With these challenges unique to our proposed core target sites, coupled with the difficult nature of finding protein/protein interaction inhibitors in general (supra), our application of detecting internal ligands would not have been, and was not obvious.

7. "The NMR method taught by Feisk [sic] is one of the most sensitive methods that detect the interactions between a protein and a small molecule … [A skilled person would] be motivated to use the most sensitive method of detecting the interaction, i.e., NMR." Action, bridging p. 2-3.

Persons with expertise in biophysical measurements of protein/small molecule interactions know that NMR spectroscopy is actually one of the least sensitive methods to look for such interactions. This is fundamentally rooted in the small energy separation between the ground and excited states that are probed by NMR spectroscopy – a small separation means small population differences, translating into poor sensitivity.

Despite recent advances in NMR magnet and probe technology, NMR still has a serious sensitivity disadvantage compared to alternatives. Practically, this means that NMR-based

screens looking for ligand-induced changes in protein signals generally require orders of magnitude more protein than competing technologies:

NMR:
typical sample: >100μM concentration, >300μL volume
= >3mg of 10kDa protein per sample
96-well thermal shift assays:
typical sample: 10μM protein concentration, 50μL volume
= 0.05mg of 10kDa protein per sample
384-well plate-based assay (e.g. PerkinElmer - AlphaScreen)
typical sample: 100nM concentration, 30μL volume
= 0.0003mg of 10kDa protein per sample

Furthermore, our protein-detected NMR assays require isotopically-labeled protein (usually ^{15}N or ^{13}C), and the only way to economically produce these proteins is by bacterial expression – *in vitro* or eukaryotic alternatives for these types of labeling are cost-prohibitive, even for large pharmaceutical companies. In addition, samples must be stable at relatively high concentration (>100μM) for extended periods of time (~ overnight) in 2-5% DMSO, should be < 30kDa, not prone to aggregation, etc.

Of course as an expert in the field, I appreciate that NMR has certain advantages. One of these, which I suspect the Examiner may be referring to in this context, is that NMR can uniquely detect low affinity binding (K_d > 1 mM, for example) as it is less prone to issues with background signals from high concentrations of free ligands compared to other methods. I also appreciate the fact that NMR methods give us resolved signals from a relatively large number of sites within a protein, allowing us to obtain some information about ligand binding site location(s) within a protein.

But even under the best of circumstances, NMR analysis is discouraged by its inherently poor sensitivity and the need to generate hundred milligrams of isotopically-labeled, stable protein. Here, it is flat-out contraindicated by the pre-confirmed absence of any NMR-apparent a priori formed ligand cavity.

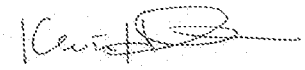
8. "Thus, one of ordinary skill in the art would not have been discouraged from using Feisk's [sic] method because the presence or absence of some NMR peaks indicating the absence or presence of a ligand-binding site." Action, p. 3.

The Examiner discusses the effect of dynamics on NMR spectra of proteins. He is right that these effects can lead to the disappearance of peaks in spectrum of a target, but these would discourage a practitioner in the field from proceeding with further studies of a protein target. The lack of peaks in this way is highly correlated with difficulty in further analysis and screens. One skilled in the art would not proceed with screening a sample using a certain method when that method does not provide the data needed to establish if ligands are binding or not.

9. "The major advantage of the NMR method over any other screening method is that it observes the binding of the small molecule directly to the target protein in its native environment, i.e. in aqueous solution. … There is no reason to believe that the most abundant conformation in solution which is observed by NMR is the most relevant conformation for binding a small molecule or a large target molecule." Action, p. 3

These sentences are inconsistent. The first argues that we should be using NMR methods for ligand screening so that we can work in a "native environment"; the second then argues that there is no reason to expect that the "most abundant" form of the protein under these conditions should be competent to bind ligand. This defies logic – why would I use a screening method that would inherently sacrifice sensitivity by having only a small fraction of the protein in a ligand-binding conformation? Either the NMR experiment is being performed under native conditions – in which case the dominant structure will tell us something that is worth screening – or the NMR experiment is not being performed under these conditions and its use is contraindicated. Here, NMR-based analysis of core ligand binding is flat-out contraindicated by the pre-confirmed absence of any NMR-apparent a priori formed ligand cavity.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application and any patent issuing therefrom.



Date: Apr 10, 2008

Professor Kevin H. Gardner